NOTES ON THE PATHOLOGICAL CHANGES IN THE ORGANS OF BIRDS INFECTED WITH HÆMOCYTOZOA

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(From the Clinical Laboratory of the Johns Hopkins University and Hospital)

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Plates X and XI.

Several authors have given brief descriptions of deposits of pigment found by them in the tissues of birds which had for some length of time been infected with hæmocytozoa. Danilewsky, in his "Parasitologie Comparée du Sang," in 1889, speaks of the changes in the hæmopoietic organs and describes macrophages containing pigment masses, parasitiferous and shrunken red corpuscles and the remains of parasites. Again, in 1890, he describes the blackening of the spleen and the enclosure of pigmented cells in large macrophages. He found also similar changes in the bone-marrow.

Kruse (2) in the same year drew attention to the dark color of the spleen, although, owing to his lack of familiarity with the anatomy of birds, he was unable to decide whether or not the organ was enlarged. He found pigment enclosed in the large clear cells in the capillaries of the spleen, liver, lung and bone-marrow; he regarded this as an evidence of the acuteness of the process, since he argues that in a chronic infection the pigment would probably all be contained in fixed cells.

Labbé (1) in 1894 speaks also in a general way of the gross changes in the organs of infected birds.

Our own observations on the pathological changes in the organs of birds infected with hæmocytozoa are based on the results of a study of the tissues of those birds whose blood has been described by Mr. Opie in the preceding paper (p. 79). In a series of about 125 birds autopsies were made in all cases in which infection was found, and for the sake of control in a few which were free from infection. In

a number of cases, of which brief accounts are given at the close of this article, the tissues were examined microscopically.

From these descriptions it will be seen that the conditions resulting from infection with the so-called "Halteridium" and the "Proteosoma" are, roughly speaking, identical. The slight differences will be brought out in the following description of the minute appearances of the various organs.

Liver. The capillaries are not as a rule dilated. In a few instances, however (No. 31), we have observed them in places to be crowded with infected corpuscles which seemed to completely occlude the lumen. Isolated infected corpuscles are seen scattered in the capillaries throughout the liver, being more numerous in the larger vessels. Many of these are well-preserved, but according to the conditions under which the tissues were hardened, the organisms have been, in greater or less proportion, extruded from the corpuscles and lie in the capillaries as pigmented spheres, accompanied by the free nuclei of the red corpuscles. The organisms in the tissues take on a pink stain with hæmatoxylin and eosin, and present a somewhat granular appearance.

The larger masses of pigment seen in the liver tissue are contained in large cells, which vary greatly in size, shape and staining properties (Plate X, Fig. 2). They are very similar to the macrophages described as occurring in the organs from cases of malaria in human beings, and are probably analogous to these. In the liver of other animals essentially identical structures have been found acting as store-houses of pigment; the previous descriptions of their occurrence in birds in hæmocytozoan infections have already been referred to. These cells are round, ovoid or irregular structures, sometimes large enough to completely block the lumen of a capillary. That they are really in the capillaries seems to be definitely proven by some of our sections, and with this view most recent writers agree. Ponfick (12), however, in his article on the "Fate of Pigment in the Animal Body," says that in the liver of animals into whose blood cinnabar has been injected, cells similar to these and containing the cinnabar granules are to be found in the lymphatics of the vessel sheaths or, at any rate, outside the blood-vessels.

He finds also that the endothelial cells of the capillaries take no part in the storing up of pigment, a conclusion quite contrary to that arrived at by us from our observations in birds.

. The protoplasm of the macrophage may remain clear and unstained; in other cases it takes on a lilac color in specimens stained in hæmatoxylin and eosin. The nucleus is always vesicular, varying in shape, being sometimes quite distorted, but oftener rounded. In some instances two nuclei have been found. Of all the varied contents of these cells the most striking is the pigment which occurs in at least two distinct forms, as follows: (1) The pigment formed by the organism—blackish-brown granules of fairly uniform size and shape, which occur separately or grouped together in spherical or irregular masses of various sizes, sometimes, apparently, far too large to be contained in a cell. (2) Straw-colored pigment, probably derived directly from the remains of the hæmoglobin which is set free into the blood stream when an infected red corpuscle breaks down and liberates the contained organism. This occurs in irregular particles, sometimes aggregated into masses similar to those just described. Although these sometimes form the only contents of the cell, many of the masses found crowding the macrophages are made up of both varieties of pigment packed together.

In other cases the large cells are filled with small masses of a fairly uniform size, sometimes irregular in outline, sometimes so closely packed together as to take on a polyhedral shape. At first sight they appear yellowish-brown, but on closer inspection are seen to be made up of a faintly colored ground substance with refractive brown granules of pigment—they are in fact shrunken parasites.

Neumann (13) and Bignami (8), in speaking of the varieties of pigment found in the organs in chronic malarial infections, recognized the fact that not only does the malarial pigment give no reaction to the ordinary tests for iron (such as Perl's reaction with ferrocyanide of potassium and hydrochloric acid or the ammonium sulphide reaction), but that, after they have been deposited in the tissues for a certain time, the iron-containing compounds derived from the hæmoglobin also cease to react. This may explain the fact that, in speci-

mens of birds' tissues which were treated with ferrocyanide of potassium, although a hazy blue stain was obtained in the cells and about the pigment, none of the fragments gave a definite blue reaction.

Treated with ammonium sulphide the pigment formed by the organisms is entirely unchanged, while in most cases some of the straw-colored variety is blackened. If, however, after the treatment with the sulphide the section be washed in water and exposed to the air, the blackened pigment becomes decolorized to a certain extent (cf. Quincke (14)), and the malarial pigment is brought out in relief.

Besides these various forms of pigment the macrophages frequently contain infected red corpuscles, which are sometimes unchanged, sometimes shrunken. In others the remains of such corpuscles form a mere debris, consisting generally of the shrunken bodies of the parasites, and finally of the broken-down remains of these with free pigment scattered in the protoplasm. The pigment is often arranged in radiating lines; sometimes it appears to lie in vacuoles. These, however, may be artefacts.

Not only the macrophages but also the endothelial cells of the capillaries may be seen to contain pigment masses (Plate X, Fig. 1). This pigment appears usually in small quantities and occurs at the poles of the nucleus. Occasionally, however, the cell is greatly distended with large pigment masses.

We are still uncertain as to the presence of pigment or parasites in any cells corresponding to Kupffer's cells in man. Many large mononuclear cells, with deeply staining nuclei and protoplasm, which takes on a lilac coloring, are found in the capillaries. Whether these can take up pigment and are to be classed with the macrophages proper we have not determined. On staining with acid fuchsin an occasional white blood corpuscle, of the class which we have for convenience termed rubinophilic cells,* may be found in a greatly swollen condition and containing pigment masses. This is, however, rather the exception, inasmuch as numerous examples of such cells are seen throughout the tissues quite free from pigment or other foreign substance.

* See page 113.

The liver cells themselves have not been observed to be phagocytic, but a very considerable pigmentation is noticeable in one or two cases (Nos. 103 and 111). Here the liver cells present a ragged vacuolated appearance and contain numerous fine straw-colored granules. On applying the ferrocyanide of potassium and hydrochloric acid or ammonium sulphide stains for iron, a sharp iron reaction is obtained and the liver presents the appearance of a close-meshed network of blue or black lines of pigment granules, according to the method used. In one liver (No. 3) there was extensive fatty degeneration.

As to the effects of the organisms or their products on the tissues but little that is definite can be stated. Necroses have been observed in the organs of several specimens, but their dependence upon the presence of the hæmocytozoa cannot be said to be proven.

We have observed two forms of focal necrosis:

(a) The first, found in the owl (No. 101), in which there was an intense septicæmia, is probably due to the presence of the bacteria. These necroses are described in the account of the autopsy (p. 115).

(b) The necroses of the second variety were found in the liver and, in a modified form, in the spleen of birds with proteosoma infection. They appear to be associated with the presence of certain oval bodies which seem to bear no close relation to the proteosoma itself (Plate X, Figs. 3 and 4). The foci in the liver are small and discrete. In the affected spots there is a complete loss of liver cells as such in the area, the centre of which is occupied by a mass of pink-staining material, which contains fragmented, deeply staining nuclei, with the debris of cells and occasional macrophages enclosing pigment and parasites (Plate XI, Fig. 1).

Among these there are often to be seen cells containing small oval bodies just about the size of the nucleus of a red corpuscle. These bodies take a general lilac color and show in their centres a ring-shaped structure which stains rather deeply with hæmatoxylin. These ovoid structures may occur free in the midst of the debris of cells and nuclei, but are more often contained in cells, where they may be seen in groups of two or more, or in larger collections of from twelve to twenty arranged with the pointed end turned to the centre and calling to mind

the appearance presented by the segmenting bodies of a malarial parasite (Plate X, Figs. 3 and 4).

These groups contain no pigment in their interstices and, from their size and general appearance, give rise to the idea that possibly they represent the segmenting stage of a protozoan parasite. This is of course only an hypothesis, and until further material is obtainable the question of their exact nature must remain undecided. Their distribution is not limited exclusively to the neighborhood of the necroses; they may be seen contained in cells elsewhere in the capillaries. They will be referred to later in discussing the spleen.

Spleen. Kruse (2) has described the changes in the spleen in infected birds. He finds that it is of a greyish-black color and contains masses of pigment within colorless cells distributed essentially in the vessels. Judging from the bright color and the great size of the granules, he believes that only one part of this pigment corresponds to that formed by the parasite. The red corpuscles taken up by the leucocytes contribute to the pigment formation, but whether the parasite first kills the corpuscle and the two are then engulfed, or whether the leucocyte swallows both alive, remains a matter of doubt.

Owing to certain peculiarities in the distribution of the pigment in the spleen it is desirable to consider certain details in the normal anatomy of the spleen of birds before entering upon a description of the pathological changes. Wilh. Müller (6) and A. J. Whiting (7) have given the most satisfactory accounts of the structure of the spleen of birds; their observations differ in no essential points from those made by us. The capsule, at least in the smaller birds, possesses no trabecular system; the vessels, however, are surrounded by a certain amount of connective tissue. In the neighborhood of these masses of connective tissue the pigment is especially deposited. At the points of branching of many arterioles spherules of lymphoid tissue are seen placed laterally, sharply marked off from the adjoining tissue by a double row of elongated cells, which Whiting considers to be muscular, but which, since they sometimes take up pigment, seem to us to resemble rather endothelial cells. These do not occur in sparrows.

The further prolongations of the arterioles show here and there

changes in the nature of their endothelium, the nuclei of which become large and vesicular. In these places the adventitial layer becomes greatly thickened so as to form an ellipsoidal mass of pink-staining tissue, in which are embedded large vesicular nuclei. Associated with these structures there is a considerable amount of lymphoid tissue poor in capillaries, the round cells of which merge gradually into the large cells of the pulp proper, which runs in bands among the spherules and ellipsoids. These pulp bands are made up of wide capillary spaces which anastomose freely in a network of large pulp cells, characterized by large vesicular nuclei of round, oval or polyhedral form, and with slightly opaque protoplasm which takes a pink stain. These cells are most closely aggregated at the margins of the bands of pulp, where in hæmocytozoan infections they become conspicuous from their actively phagocytic properties. Sometimes, as in case No. 101, they become so loaded with pigment that the pulp bands appear as a black network on section of the spleen (Plate XI, Fig. 2). They exist in numbers directly under the capsule, where again they may contain a conspicuous collection of pigment. The extent of the pigmentation varies greatly, as will be seen from the autopsy reports. The nature of the deposit, however, and its distribution is similar in all, the pigment resembling exactly that described as occurring in the liver, and giving the same staining reactions.

The proportion of the varieties of pigment differs to a certain extent in the different specimens; for example, in No. 104 the pigment is mostly yellow, while in Nos. 5 and 103 it is mainly of the dark variety; this is, however, possibly dependent on the duration of the process, the yellow pigment being, as Bignami has suggested, changed by a local reaction into a dark material which does not respond to ordinary tests for iron. Almost all of this pigment is contained in the large cells mentioned above as forming the groundwork of the vascular bands, and most closely arranged along the margins of these pulp bands, directly under the capsule and in the neighborhood of the large vessels. As a direct result of this arrangement we have a network of pigmented cells, as shown in Plate XI, Fig. 2.

As to the exact nature of these cells we cannot be quite certain; but since our observations agree with those of Müller, who finds no defi-

nite endothelial lining to the capillary blood spaces, it seems probable that these cells, which themselves form the limits of the blood spaces, are to be considered as essentially endothelial in nature. Their form, when they have acted as phagocytes, is very similar to that of the macrophages seen in the capillaries and larger vessels of the spleen, liver and other organs. Distended sometimes to a great size and crowded with pigment balls, pigment granules, infected red corpuscles and debris of various sorts, their nuclei swell and become pale, while the protoplasm often becomes clear. Deposits of pigment also occur in the elongated cells which outline the spherules, as a result of which they sometimes become greatly distended.

Degenerative changes have also been observed in the spleen. Those occurring in connection with the bacterial infection in case No. 101 are identical in character with the necroses observed in the liver of that case.

In two cases (Nos. 103 and 111) a considerable fragmentation of nuclei was noted, more especially in the lymphoid spherules, where the bulk of the nuclei are represented by very deeply staining dots. The cells have lost their contour and the whole spherule has a degenerated appearance.

Quite a different picture is presented in the spleens of Nos. 5, 80, 31, and some others where there are well-defined annular hyaline deposits which stain bright pink. These rings often enclose groups of large macrophages containing yellow pigment. In the cases of proteosoma infection there are found also in the spleen the peculiar ovoid bodies referred to above in the description of the liver, although none of the above lesions can be definitely ascribed to their presence. These present the same appearance as in the liver, excepting that they are more frequently arranged in beautiful symmetrical groups as in a segmenting organism. These groups vary much in size, the segments numbering from 5 to 20 or more; as in the liver, they occur within large cells, which here may be definitely identified as cells of the splenic pulp. They are most numerous in the neighborhood of the ellipsoids and generally in the less richly vascular portions of the spleen. In case No. 43 there are areas in which almost every cell contains a group, some cells containing even two. It is not improbable that some light

upon the nature of these bodies may be obtained later by aspirating the spleen of infected birds during life.

Bizzozero (5) has given a very good account of the structure of the *bone-marrow* in birds. It is unnecessary, however, at the present time to review his work. All writers upon the subject who have so far referred to the pigmentation of the organs in birds, have especially noted the dark color of the bone-marrow. Danilewsky (3) describes large protoplasmic bodies containing pigment and fragmented red corpuscles, and again (9) numerous "macro-melanophages." Labbé (1) and Kruse (2) find pigment in the capillaries of the bone-marrow in virulent cases.

Our own experience has not impressed us with the intensity of the pigmentation in the bone-marrow. Unfortunately, in case No. 101, where there was a most intense infection and where the other organs were deeply pigmented, the bone-marrow was lost; but in the succeeding cases where it was carefully studied no accumulation of pigment was observed, and but few organisms as such were found in the capillaries. In case No. 3 numerous organisms were seen, but no pigment blocks, and it was only in Nos. 81 and 31, where smears were made from the bone-marrow, that large pigmented cells were found in moderate numbers. These with their contents were in most respects similar to the large phagocytic cells seen elsewhere, with the exception that the pigment was in smaller masses.

But little remains to be said of the *visceral changes*. The brain in all our cases was quite normal; of course intra- and extra-corpuscular organisms were occasionally seen in the capillaries, but they were never collected in masses sufficient to occlude the lumen of the vessel. There was no pigmentation of the tissues except that a few doubtful pigment granules were situated at the poles of the nuclei of some of the endothelial cells in the capillaries in case No. 31.

Organisms were found in the capillaries of the lungs, but beyond inspired carbon particles no further pigmentation of the lung was to be made out. This is contrary to the experience of Kruse (2), who always found pigmentation of the lungs. This coal pigment is of a dead black color, readily distinguishable from either of the forms of pigment described as the result of the infection. Chemically it is not

acted upon by any of the reagents employed to demonstrate the presence of iron. It is distinguishable in the neighborhood of the bronchi and air sacs and is evidently in close relation to the lymphatics.

The stomach (proventriculus) and intestine were quite normal in all the cases with the exception of the presence of a few organisms in the vessels of the mucosa.

The kidneys showed occasionally a few intravascular organisms, but were otherwise normal.

The pancreas, thyroid, heart and muscles showed nothing abnormal.

Phagocytosis, which apparently bears a close relation to all this pigmentation of the organs, has not been very satisfactorily studied in the blood. Labbé (1) denies the existence of an active protective phagocytosis; wherever he found infected corpuscles within the leucocyte the normal elements were associated with them. He admits, however, that parasites are engulfed and destroyed by the phagocytes and more especially by those of the hæmopoietic organs.

Kruse (2) has observed the engulfing by the leucocytes of the pigment left by the degenerated flagellated forms.

Danilewsky (3) followed carefully the changes seen in the infected red corpuscles on being engulfed by an active phagocyte. He finds that the stroma of the red element is first slowly digested away, leaving the intact organism, and that the organism then loses its sharpness of contour and is finally lost to view, leaving only the pigment.

The morphology of the leucocytes as given by Bizzozero (4) is briefly as follows:

(1) Cells 6.5 to 7.8μ in diameter with rather large polymorphous nucleus and clear protoplasm, in which are contained numerous highly refractive fusiform rodlets which take on an eosin stain.

(2) Cells of the same size with similar nucleus and protoplasm, but with small spherical eosinophilic granules—cells almost like human eosinophiles.

(3) Small leucocytes with large spherical nucleus and homogeneous protoplasm.

(4) Large leucocytes 6.7 to 8.3μ in diameter, spherical or oval, with large nucleus and opaque finely granular protoplasm.

Of these we have found that the first are the more common, corres-

ponding apparently in numbers to the polymorphonuclear neutrophiles in human blood. For the sake of brevity in description it has seemed to us advisable to adopt some special term to distinguish this variety of cell. The greater richness with which these particular granules stain with acid fuchsin has suggested to us that the name *rubinophile* might not be unfitting, and it is by this term that we shall hereafter refer to them. They are evidently the chief agents concerned in phagocytosis in the circulating blood. After watching for a long time, we have observed that they sometimes engulf degenerated organisms and present then the same appearance as the pigmented leucocytes in human blood. In the fresh slide we have several times observed the leucocyte, after partly surrounding the organism, to leave it in a still further disintegrated condition and wander away. It appeared to engulf the protoplasmic remains and leave the pigment free in the plasma. Even in such an intense infection as that which had occurred in the owl (No. 101), where several organisms could be seen in every field, we found no pigmented leucocytes in the fresh blood; nor have we met with pigmented rubinophilic leucocytes in the vessels of the tissues except in one or two instances, although in clots of blood taken from the aorta we have occasionally found macrophages loaded with pigment and the detritus of organisms. The exceptions referred to were demonstrated in the liver of case No. 101 by staining with acid fuchsin. In these specimens two rubinophilic cells very much swollen and containing masses of pigment were found in the capillaries.

In these changes there is seen a marked general resemblance to those occurring in the organs of human beings suffering from malarial infection, changes which have been so accurately described by Barker (10). The chief point of difference is in the behavior of the white blood corpuscles, which appear here to play but little part in the changes in the tissues. In no instance have we observed the inclusion of the leucocyte within a macrophage and, although in the liver of case No. 81 there were great accumulations of rubinophilic cells, not one is seen to contain pigment or the remains of an engulfed parasite. The intimate nature of the different pigments here found and their

relation to that found in the tissues of human beings with malarial infection merits careful chemical study.

In conclusion I wish to express my thanks to Dr. Thayer, to whom I am indebted for many kindnesses and much substantial assistance in this work, which was undertaken at his suggestion and continued under his supervision; also to Dr. Barker, who has examined my preparations and given me many valuable suggestions.

The main points in the results of the autopsies may be briefly summarized as follows:

(No. 3.) *Passer domesticus* (English sparrow); Halteridium infection. *Liver* mottled, dark reddish brown; shows fatty degeneration and slight pigmentation.

Spleen greyish white; microscopically, shows few organisms and very little pigmentation.

(No. 5.) *Passer domesticus;* Halteridium infection. *Spleen* enlarged; bluish black. Microscopically, shows bands of black pigment corresponding with the pulp bands and an extensive subcapsular deposit. The dense pigment masses are intracellular. *Liver* somewhat pigmented; the large cells containing pigment lie in the capillaries. The other organs normal in appearance.

(No. 31.) *Passer domesticus;* Proteosoma infection. *Liver* large, homogeneous, dark brown. Microscopically, the capillaries of the liver are often found crowded with infected corpuscles. There are areas of focal necrosis with central hyaline deposit, loss of liver cells and many distorted nuclei (Plate XI, Fig. 1). In the capillaries surrounding these, but much oftener in the large intracapillary cells, are found ovoid bodies which have been described above. *Spleen* enlarged, black; much pigment on teasing; densely pigmented in a characteristic way and presenting annular areas of hyaline change. Numerous groups of the ovoid bodies may be found here also.

(No. 43.) *Passer domesticus;* Proteosoma infection. *Liver* large. *Spleen* 20 x 3 mm.; black. There is extensive pigmentation of both liver and spleen, and great numbers of the above-mentioned ovoid bodies are found in both organs (Plate X, Figs. 3 and 4). Post-mortem changes have masked the effects on the tissues.

(No. 80.) *Agelaius phoeniceus* (blackbird). *Liver* dark in color; slightly pigmented. *Spleen* enlarged, black. There is moderate pigmentation and very numerous regular symmetrical groups of ovoid bodies are to be seen.

(No. 81.) *Passer domesticus;* Halteridium infection. *Liver* light brownish red. Microscopically shows areas crowded with leucocytes and round granular eosinophiles; pigmentation slight. *Spleen* greyish brown; not enlarged; some pigment seen on teasing. Much pigment in bone-marrow.

(No. 101.) *Bubo virginianus* (horned owl); Halteridium infection. Bird lived some time with a broken wing and with shot in its lungs and other organs. *Liver* chocolate brown; microscopically deeply pigmented; capillaries contain numerous macrophages and other phagocytic cells. *Spleen,* 1.5 x .7 cm. (twice normal size); microscopically shows most intense pigmentation corresponding to the margins of the pulp bands, so that on section it presents a coarse network of black lines (Plate XI, Fig. 2). Bone-marrow unfortunately not preserved. In all the organs there are focal necroses in which are seen masses of bacilli of a rod-like form, staining weakly and occurring sometimes in threads. They are also found distributed in the capillaries and unassociated with necroses. Cultures were not made. This septicæmia was probably a terminal infection.

(No. 103.) *Corvus americanus* (crow); Halteridium infection. *Spleen* dark red; microscopically shows areas of cellular degeneration with fragmentation of the nuclei. These are limited to the spherules of lymphoid tissue. *Liver* normal in size and color; pigmentation not extensive; a few rubinophilic leucocytes are seen to contain pigment in the liver capillaries.

(No. 104.) *Passer domesticus;* Proteosoma infection. *Liver* brownish; microscopically, definite focal necrosis associated with the ovoid bodies, as in case No. 31. The pigment is in small dense masses and moderate in amount. *Spleen* large; 10 x 2 mm.; bluish red in color; ovoid bodies similar to those in the liver are to be found also in the spleen.

(No. 111.) *Corvus americanus;* Halteridium infection. Organs looked fairly normal. *Spleen* rather dark in color; microscopically, pigment is not abundant and has the usual distribution; occasional areas of degeneration in the lymphoid nodules. The liver cells, as in No. 103, are vacuolated and contain much fine yellowish pigment, which gives the iron reaction. Bone-marrow dark reddish brown; no pigment masses and very few organisms are to be seen.

(No. 119.) *Corvus americanus;* Halteridium infection. *Spleen* blackened; organs otherwise of normal appearance. *Liver* shows slight pigmentation; few macrophages and endothelial cells containing masses of pigment. Bone-marrow negative. There was here, as in No. 111, a coincident infection with filaria.

DESCRIPTION OF PLATES X AND XI.

PLATE X.

Fig. 1.—Portion of the liver of an infected bird showing a pigmented endothelial cell.

Fig. 2.—Macrophages loaded with pigment, etc., as seen in the spleen and liver.

Fig. 3.—From the liver of case 43, showing the intracellular ovoid bodies described p. 107.

Fig. 4.—An isolated liver cell containing a group of the ovoid bodies.

PLATE XI.

Fig. 1.—From the liver of case 31, showing a focal necrosis with loss of liver cells, which are replaced by hyaline material. In many places groups of the ovoid bodies can be seen in the necrotic tissue.

Fig. 2.—From the spleen of case 101, showing the distribution of the pigment. Focal necroses caused by bacteria are also seen in the pulp.

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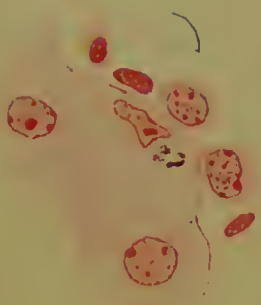

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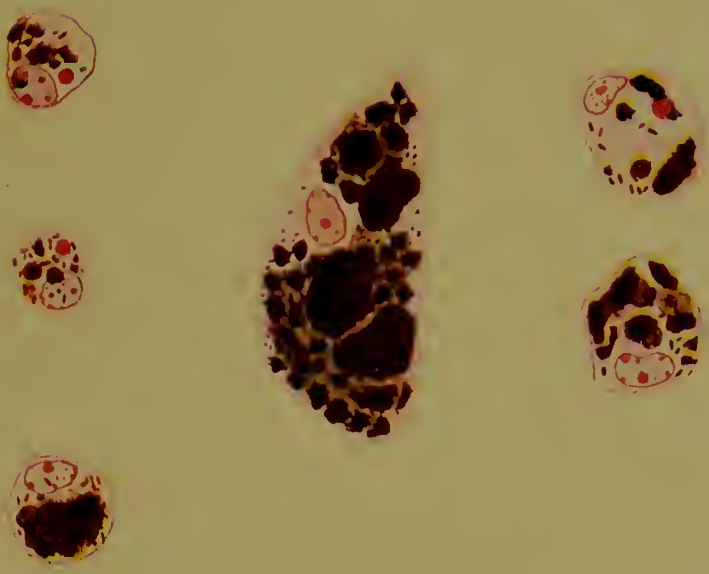

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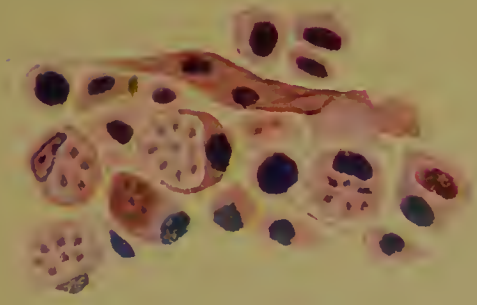

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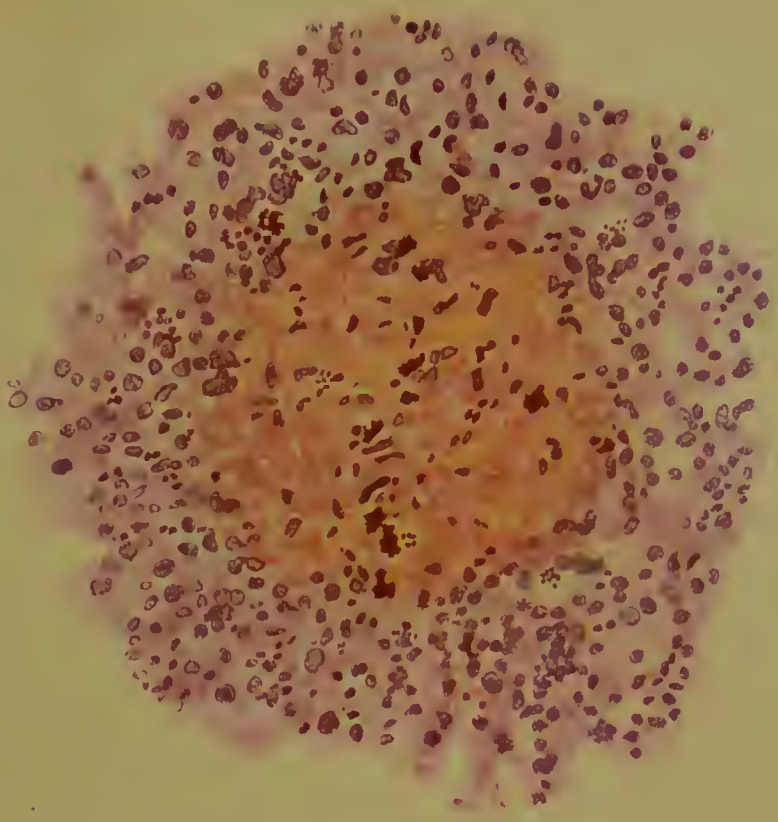


Fig. 1

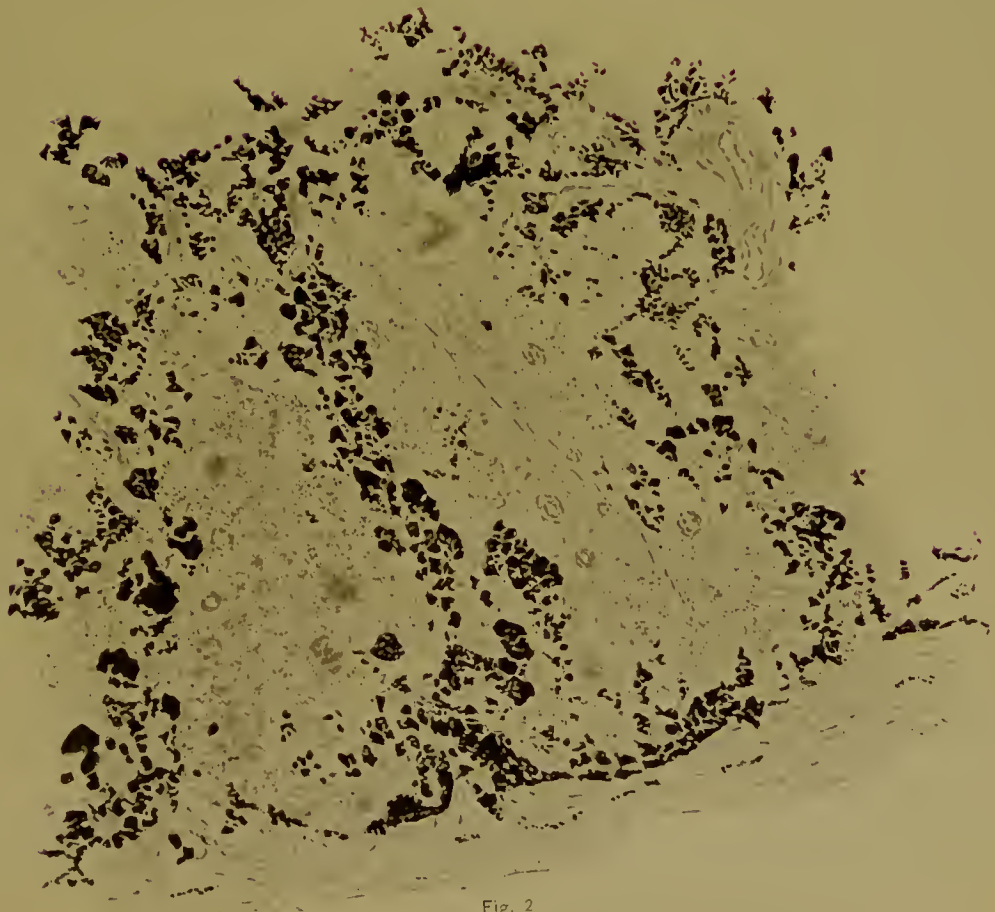
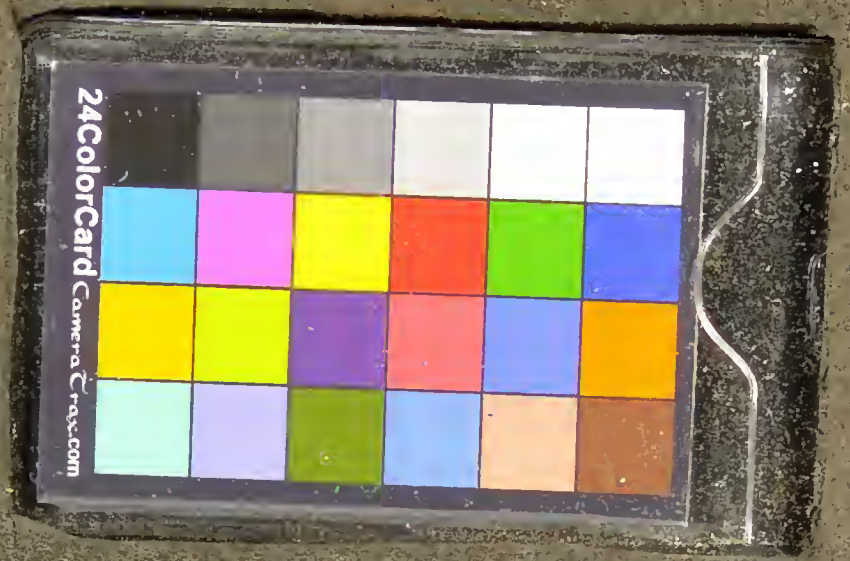


Fig. 2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